International Journal of Zoology
and Research (IJZR)
ISSN(P): 2278-8816; ISSN(E): 2278-8824
Vol. 4, Issue 1, Feb 2014, 1-6

TRANS STELLAR
• Journal Publications • Research Consultancy

CHARACTERIZATION OF SNOUT IN THE HILL STREAM FISHES; *BOTIA ALMORHAE* (TELEOSTEI: COBITIDAE), *HOMALOPTERA BRUCEI* (TELEOSTEI: BALITORIDAE) AND *SCHIZOTHORAX RICHARDSONII* (TELEOSTEI: CYPRINIDAE) OF KUMAUN HIMALAYA: A SCANNING ELECTRON MICROSCOPIC (SEM) INVESTIGATION

HOSHIYAR SINGH

Department of Zoology, S. S. J. Campus, Almora - Kumaun University, Nainital, Uttarakhand, India

ABSTRACT

The present investigation has been designed to conduct a comparative study of the functional organization of the epidermis of the snouts of *B. almorhae, H. brucei* and *S. richardsonii.* The skin of snout is scale-less in *B. almorhae, H. brucei* and *S. richardsonii* (Gray 1830) and the skin is composed of two distinctive layers; epidermis and dermis. The epidermis is both types are smooth or non-keratinized and rough or keratinized. The smooth epidermis of snout possesses epithelial cells and mucous cell apertures interspersed between the epithelial cells in these fishes, with the presence of a thick coat of mucus over the snout of *B. almorhae, H. brucei* and *S. richardsonii* that is liable to more friction when the fish swim upstream is significant. The surface of the epithelial cells is characterized by well-developed microridges, which reflect their high secretory activities in all the above fishes.

KEYWORDS: Epidermis, Snout, Epithelial Cells, Mucous Cell and Microridges

INTRODUCTION

B. almorhae are easily characterized by their elongated snouts. Snout pointed, rather short, its length equal to or shorter than half of head length, its tip separated from tuft of rostral barbels by a horse-shoe shaped ridge of skin. In *H. brucei*, snout characterized by broad and rounded, strongly depressed, sharp-edges, provided with trenchant margins, covered with small sharp tubercles. In *S. richardsonii*, snout obtuse, usually smooth, males may have tubercles. Surface architecture of snout epidermis of these three hill-stream fishes was examined by scanning electron microscopy, in an attempt to understand the structural and functional modifications in epithelia, in relation to life in torrential streams.

MATERIALS AND METHODS

The live fishes viz. *Botia almorhae* (Teleostei: Cobitidae), (approximately 5-7 inches in length) were collected from the Kosi river at Kakrighat of Distt. Nainital (elevation- 1200 m. above mean sea level), *Homaloptera brucei* (Teleostei: Balitoridae), (approximately 3-4 inches in length) from West Ramganga at Chaukhutia in Distt. Almora (elevation-1200 m. above mean sea level) and *Schizothorax richardsonii* (Teleostei: Cyprinidae), (approximately 6-8 inches in length) from the Kosi river at Hawalbagh in Distt. Almora (elevation- 1194 m. above mean sea level) Uttarakhand. The water current is very fast having the velocity between 0.5 to 2.0 m/sec. (Bhatt and Pathak, 1991) and the river bed is rocky.The fishes were transferred from the site of collection to the laboratory in well ventilated plastic containers and were kept for a period of about 5-6 days in glass aquaria having an artificially prepared rocky bed with aquatic vegetation grown therein. The aquaria were cleaned and supplied with fresh spring water on alternate days. The fishes were fed on aqua feed (tropical fish food).

To study the details of the morphological adaptations in some fishes, SEM was done. The following procedure was adopted for the preparation of the specimen for SEM.

The specimen was maintained in laboratory at 25±2^{0}C. The fishes were cold anesthetized following Mittal and Whitear, 1978, for SEM preparation. Skin fragments of snout, about 10×10 mm were cut from their body. Tissue were excised and rinsed in 70% ethanol with one change of saline solution to remove debris and then fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 over night at 4^{0}C in a refrigerator. The tissues were washed with 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30 mins.) and dried at critical point using a critical point dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using conductive silver preparation (Eltecks, Corporation, India). The samples were coated with gold using a sputters coater (JFC 1600) and examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

RESULTS

Snout epidermis of *B. almorhae*, *H. brucei* and *S. richardsonii* is of both types, smooth and rough. The smooth epidermis possesses both epithelial and mucous cells. Epidermal cells cover the skin by polygonal epithelial cells (Figure 1), epithelial cells possess microridges; in *B. almorhae* the microridges on the epithelial cells are also interesting in that they periodically seem to be expanded in the form of a villus-like structure (Figure 2). The boundaries of the epithelial cells are clearly defined (Figure 2). The mucous cell apertures occur at the border of three or four epithelial cells. The rough epidermis of snout of *B. almorhae* has small knob-like structures, the tubercles (Figure 3). Unculi are absent on the tubercles. At the apical end of each tubercle closely packed microvilli are observed (Figure 4).

In *H. brucei*, the surface architecture of the epithelial cells is, in general, uniform and characterized by short and narrow microridges, which are sometimes branched and are irregularly interwoven to form intricate and filamentous patterns (Figure 5 and 6). The boundaries between adjacent epithelial cells are demarcated by poorly defined, double rows of microridges. The mucous cell apertures are rare and occur at the border of three or four epithelial cells (Figure 5 and 6). The dorsal surface of snout epidermis of *H. brucei* is also tuberculated. These tubercles are small, wart-like or of conical shape and are generally discrete (Figure 7 and 8), conical and multicellular. The epithelial cells of each tubercle develop into unculi. The unculi are equidistantly placed and supported by epithelial cells. Polygonal outlining of the epidermal cells is seen at the base of the unculi, indicating unculi to be modified epithelial cells (Figure 9).

In *S. richardsonii*, the mucous cells, though distributed throughout the epidermis are in general, concentrated mainly in the outer layer of the epidermis often releasing their secretory contents profusely at the surface through a small pore. Interspersed between the epithelial cells, the mucous cells are distinguishable. In many cases mucus can be seen around these openings (Figure 10). Generally, such apertures occur where the boundaries of three or more epithelial cells meet (Figure 11). The surface architecture of the epithelial cells (Figure 12) is characterized by the presence of microridges; the microridges on the epithelial cells are also interesting in that they periodically seem to be expanded in the form of a villus-like and filamentous structure (Figure 12). The boundaries of the epithelial cells are well developed and clearly defined (Figure 12). The rough epidermis of snout possesses tubercles (Figure 13 and 14). The snout is tuberculated and blunt in the mature male while non-tuberculated and pointed in the female (Singh, 2003). The length of epidermal tubercles of *S. richardsonii* is approximately 128.88µm. The base of each tubercle is broad, rounded and its approximate width is 282.06µm (Figure 15).

DISCUSSIONS

The epidermal cells at the surface of the investigated fishes are composed of vertically compressed epithelial cells. It forms a continuous covering on the surface. It is interspersed with mucous cells opening at the surface. The rough epidermis possesses tubercles. A large number of tubercles are found in *H. brucei* and *S. richrardsonii* as compared to *B. almorhae.* The large number of tubercles in males indicates increasing reproductive power of the fishes. The primary function of the epidermis is to provide protection against environmental hazards. In fish, this function is mainly attributed to the gland cells which secrete their contents on the surface.

The present investigation has been designed to conduct a comparative study of the functional organization of the epidermis of the snouts of *B. almorhae, H. brucei* and *S. richardsonii*. The snout epidermis is subjected to more frictional stress as compared to the epidermis covering the general body surface as it is the first to come in contact with the water current, especially when fish swim upstream and hence a correlation has been established in relation to friction.

B. almorhae is primarily a bottom dwelling fish (like most of its relatives) and prefers a substrate composed of smooth rock, silt and gravel. Hiding places in the form of caves or plant cover are much appreciated by this fish.

In *H. brucei* the Snout is depressed and bluntly rounded. Just like other hill-stream loaches, species of *Balitora* are reported to inhabit the benthic region of fast flowing water bodies (Hora, 1932 and Chen et al., 2005).

S. richardsonii is the most common in rivers and is held in high esteem by the local people. Snow trouts are the dominant fish in the streams and rivers, with the bulk formed by *S. richardsonii.* S*chizothorax* species generally prefer rapid and pools of snow fed torrential streams, *Schizothorax* have a blunt snout. Misra, 1959 has described *Schizothorax* and *Schizothoraichthys* based on the shape of snout, with *Schizothorax* having a blunt snout and suctorial lip whereas *Schizothoraichthys* has a pointed snout and no suctorial lip, is widely distributed in the Himalayan and Subhimalayan region of the Indian-Chinese sub-continent.

Noticeable difference exhibited in the distribution of mucous cells may be considered as modifications relating to possible difference in the functional requirement at the different locations. A large number of mucous cells are present in *B. almorhae* and *S. richardsonii* a compared to *H. brucei*; it means these fishes live against high velocity currents of water. This may provide sufficient lubrication to reduce the friction between the body surface and water current and protect the epidermis from wear and tear.

It is interesting to note the dorsoventral part, which is liable to more friction when the fish unearthens the substratum is keratinized and is devoid of glandular epidermis in *H. brucei*. This is probably an adaptation to the peculiar digging mode of life of this fish as keratinized surface can better protect from wear and tear, providing hardness, durability and mechanical strength than that of glandular epidermis (Bisht and Agarwal, 2001).

The free surface of epithelial cell bears a series of microridges. Microridges are thought to be involved in the retention of mucus, in addition to facilitating the spreading of the mucus over cell surface (Sperry and Wassersug, 1976). Fishelson, 1984 related the variations in microridge patterns to locomotory activity and suggested that in faster swimming fishes, the most developed ridges served to trap mucus on the epithelial surface. Microridges have been reported to vary considerably in configuration and deposition, constituting varied patterns at different locations in different fish species and have been implicated to play variable roles. Whitear, 1990 suggested the mucogenic epidermis that forms of the microridges is related to the process of secretion of slime.

This could be considered as an adaptation to withstand mechanical stress and protect the surface of the fish, which has the characteristic habit of bottom dwelling. Furthermore, these microridges may gain a firm base and support from a dense network of fine filaments.

The abundance or dearth of the mucous cells in the epidermis may also be correlated with their mode of life (Mittal and Banerjee, 1975). Mucus is secreted in receiving the necessary stimuli from the surrounding environment, providing a sort of platform for feeble adhesion. Such neuromuscular organs have also been reported in *G. garhwali* (Johal and Rawal, 2003).

Breeding tubercles may also act as hydrodynamic or tactile stimulators of females during courtship. They are predominantly found in males and are usually prominent during the breeding season when they help males maintain contact with females during spawning. The best known are probably the breeding tubercles of Cyprinid fishes in which high numbers of tubercles have been shown to increase male reproductive success.

Kortet and Taskinen, 2004 reported that breeding tubercles might offer a workable tool for the examination of the sexual selection among Cyprinids. Appearance of tubercles in different areas of the body during breeding season has been observed in seven families of bony fishes under five orders; namely Salmoniformes, Gonorhynchiformes, Cypriniformes, Scorpaeniformes and Perciformes (Jyoti and Sharma, 2006). These breeding tubercles are local keratinizations distinctly different from normal epidermis and they facilitate contact between the individual fishes during breeding (Jyoti and Sharma, 2006). The development of breeding tubercles may take weeks rather than days and they are shed shortly spawning (Wiley and Collette, 1970).

CONCLUSIONS

Surface architecture of snout epidermis of hill-stream fishes; *B. almorhae*, *H. brucei* and *S. richardsonii* was examined by scanning electron microscopy, in an attempt to understand the structural and functional modifications in epithelia, in relation to life in torrential streams.

ACKNOWLEDGEMENTS

A special word of hearty thanks to Mr. M.P. Singh, SEM specialist, College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, (Udham Singh Nagar) for helping me to carry out the Scanning Electron Microscopy involved in the study. I am very much beholden for the necessary facilities he offered without any reservation.

REFERENCES

1. Bhatt, S.D. & Pathak, J.K. (1991). Streams of Great Mountain are Physiographic and Physiochemistry. In: Ecology of the mountain water. Ashish pub. House New Delhi, 43-58.
2. Chen, X.Y., Cui, G.H. & Yang, J.X. (2005). *Balitora nantingensis* (Teleostei: Balitoridae), a new hill-stream loach from Salween Drainage in Yunnan, southwestern China. The Raffles Bulletin of Zoology, 13, 21-2 6.
3. Bisht, I. & Agarwal, S.K. (2001). Histophysiology of snout epidermis of hill-stream fish: A comparative study. Biochem. Cell. Arch., 1, 1/2, 9-23.
4. Fishelson, L. (1984). A comparative study of ridge-maizes on surface epithelial cell/membrances of fish scales (Pisces; Teleostei). Zoomorphologie, 104, 231-238.

5. Hora, S.L. (1932). Classification, bionomics and evolution of homalopterid fishes. Memoirs of the Indian Museum (Calcutta). 12, 262-330.

6. Johal, M.S. & Rawal, Y.K. (2003). Mechanism of adhesion in a hill-stream fish; *Glyptothorax garhwali* Tilak, as revealed by scanning electron microscopy of adhesive apparatus. Current science, 85, 1273-1275.

7. Jyoti, M.K. & Sharma, A. (2006). Fishes, aid to collection preservation and identification. Daya Publishing House, Delhi, 179.

8. Kortet, R. & Taskinen, J. (2004). Parasitism, condition and number of front head breeding tubercles in roach; (*Rutilus rutilus* L.). Ecol. Freshw. Fish, 13, 119-124.

9. Misra, K.S. (1959). An aid to the identification of common commercial fishes of India and Pakistan. Res. India Mus., 5, 1-320.

10. Mittal, A.K. & Benerjee, T.K. (1975). Histochemistry and the structure of the skin of a murrel: *Channa striatus* (Bloch, 1977) (Channiformes: Channidae). I. Epidermis. Can. J. Zool., 53, 833-843.

11. Mittal, A.K. & Whitear, M. (1978). A notes on cold anaesthesia of poikilotherms. J. Fish. Biol., 13, 519-520.

12. Sperry, D.G. & Wassersug, R.J. (1976). A proposed function for microridges on epithelial cell. Anat. Rec., 185, 253-258.

13. Whitear, M. (1990). Causative aspects of microridges on the surface of fish epithelia. J. Submicrose. Cytol. Pathol., 22, 211-220.

14. Wiley, M.L. & Collette, B.B. (1970). Breeding tubercles and contact organs in fishes: their occurrence, structure and significance. Bulletin of the American Museum of Natural History, 143, 145-216.

15. Figure 1: SEMPH of *B. almorhae* of showing polygonal epithelial cells and mucous opening (Marked by arrow) (Scale bar- 10µm).

16. Figure 2: SEMPH of *B. almorhae* showing villus-like microridges (Marked by arrow) (Scale bar- 5µm).

17. Figure 3: SEMPH of the snout epidermis of *B. almorhae* showing epidermal tubercles (Marked by arrows) (Scale bar- 500µm).

18. Figure 4: SEMPH of the snout epidermis of *B. almorhae* showing closely packed microvilli at the apical surface of the tubercles at high magnification (Scale bar- 10µm).

19. Figure 5: SEMPH of *H. brucei* of showing polygonal epithelial cells (Marked by arrow) (Scale bar- 10µm).

20. Figure 6: SEMPH of *H. brucei* of showing filamentous microridges and mucous openings (Marked by arrow) (Scale bar- 10µm).

21. Figure 7 and 8: SEMPH of the snout epidermis of *H. brucei* of showing wart-like or conical tubercles (Marked by arrow) (Scale bar- 100µm).

22. Figure 9: SEMPH of *H. brucei* showing polygonal epithelial cells and unculi on the tubercles (Marked by arrow) (Scale bar- 10µm).

23. Figure 10 and 11: SEMPH of *S. richardsonii* showing polygonal epithelial cells and mucous openings (Marked by arrows) (Scale bar- 50 µm and 10 µm).

24. Figure 12: SEMPH of *S. richardsonii* showing both villus-like and filamentous microridges (Marked by arrows) (Scale bar- 5 µm).
25. Figure 13: SEMPH of *S. richardsonii* showing epidermal tubercles (Marked by arrows) (Scale bar- 500 µm).
26. Figure 14: SEMPH of *S. richardsonii* showing single epidermal tubercles (Scale bar- 100 µm).
27. Figure 15: SEMPH of *S. richardsonii* showing broad base tubercles at high magnification (Scale bar- 100µm).

APPENDICES

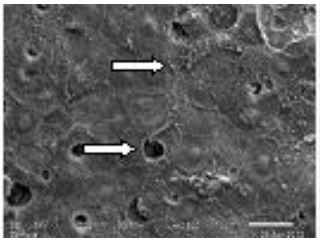

Figure 1

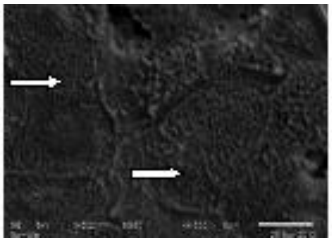

Figure 2

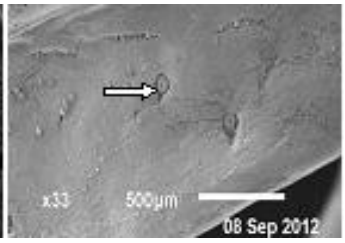

Figure 3

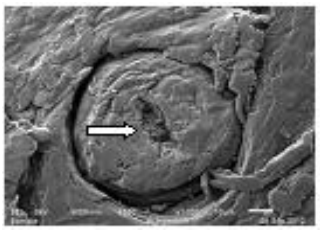

Figure 4

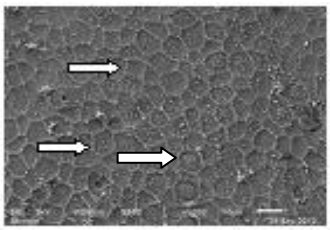

Figure 5

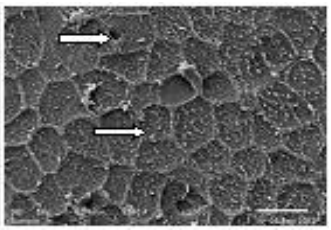

Figure 6

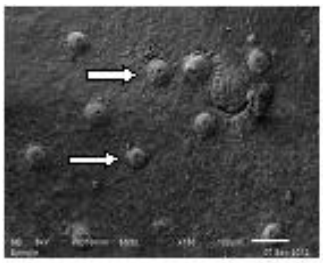

Figure 7

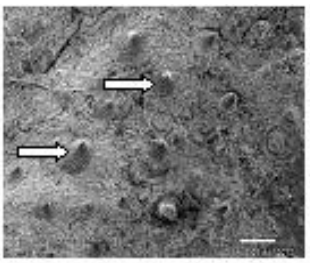

Figure 8

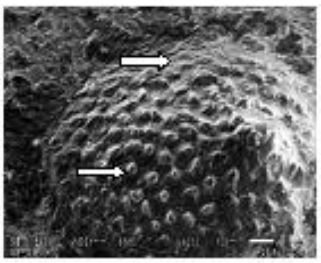

Figure 9

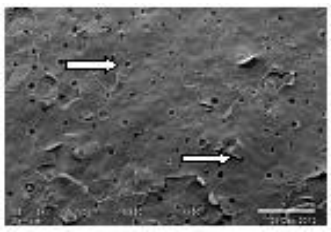

Figure 10

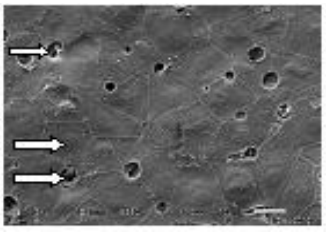

Figure 11

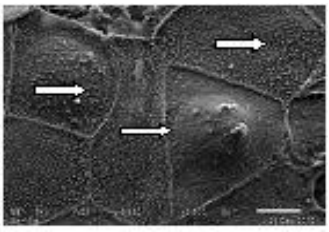

Figure 12

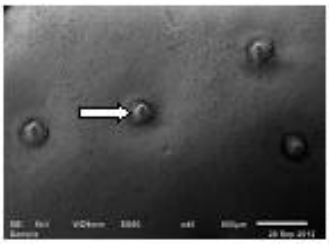

Figure 13

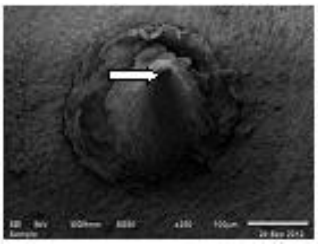

Figure 14

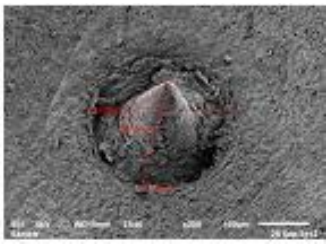

Figure 15